SPECIAL COMMITTEE APPOINTED BY THE ROYAL SANITARY INSTITUTE.

DISINFECTANT STANDARDISATION COMMITTEE.

In the course of their consideration of the above subject, the following Report of Experiments made on behalf of this Committee has been submitted to them by Lieut.-Col. R. H. FIRTH, F.R.C.S., D.P.H., R.A.M.C., *and* Professor ALLAN MACFADYEN, M.D., B.SC., F.I.C.

FOR the further elucidation of the question as to what method is best adapted for the routine standardisation of disinfectants, your Committee requested us to carry out additional experiments to those which have already been submitted.

The accompanying schedule presents the results of a large number of observations which have been made under our supervision by Major C. E. P. Fowler, R.A.M.C., in the hygiene laboratory of the Royal Army Medical College on behalf and at the expense of The Royal Sanitary Institute.

The general plan of these experiments has been to test the comparative value of three methods, namely "the Garnet," "the Thread," and the "Drop," using as test micro-organisms (1) spore bearing anthrax bacilli, (2) various strains of the *B. typhosus*, and (3) the *B. prodigiosus*. The selection of these particular varieties of bacterial life was made because they respectively represent a typical resistant pathogenic form, a typical fæcal pathogenic micro-organism, and a typical saprophytic microbe. A few observations have been made with the cholera vibrio. Further, the experiments have been carried out to determine, if possible, whether bichloride of mercury could be utilised as the standard reagent in place of phenol, as had been suggested previously, and also to test the three methods with a variety of commercial disinfectants in common use.

The "Garnet" method employed is a modification of that suggested by Kronig and Paul, and has been described already in the proceedings of your Committee. When working with bichloride of mercury, the garnets were washed with sterile saturated ammonium sulphide solution, but when other disinfectants were used, the garnets were washed only in sterile distilled water before being transferred to the broth sub-culture tubes.

The "Thread" method has been carried out in the following way:

Apparatus required:—

60 sterile watch glasses.
30 tubes of nutrient broth.
1 sterile test tube.
1 sterile glass funnel.
1 sterile filter paper.
30 sterile silk threads, one inch in length of standard calibre. The standard employed has been Pearsall's flannel embroidery, silk size No. 4 (extra stout), cream colour.
1 empty sterile Petri dish.
2 sterile Petri dishes, each containing two sterile filter papers.
Some sterile distilled water.
2 twenty-four-hour agar growths of micro-organisms under observation.

Technique.—An emulsion of the micro-organisms is made by adding 5 cc. of sterile distilled water to each of the agar slope growths and carefully scraping off the growths by means of a platinum wire loop, followed by gentle agitation. The resulting emulsions are then filtered through the sterile filter paper into the sterile test tube. The filtrate there collected is now poured into the sterile Petri dish, and 30 silk threads (of standard length and thickness) soaked in the filtered emulsion for one hour at 37° C. After soaking for an hour, each thread is transferred separately by means of sterile forceps to the two other Petri dishes, which have the filter paper covering their bottom. Excess of emulsion is thus absorbed, and the two dishes, with their contained infected threads, allowed to dry by placing them in the 37° C. incubator for 20 hours.

Diagram illustrating the working of the "Thread" Method.

Nature and Dilution of Reagent employed.	Group I. of watch glasses containing diluted Disinfectant. Period of Contact in minutes.						Group II. of watch glasses containing Water, or Sulphide for washing the threads.					
	2½	5	7½	10	12½	15						
X disinfectant 1-400 E.	o	o	o	o	o	o	o	o	o	o	o	o
„ „ 1-300 D.	o	o	o	o	o	o	o	o	o	o	o	o
„ „ 1-200 C.	o	o	o	o	o	o	o	o	o	o	o	o
„ „ 1-100 B.	o	o	o	o	o	o	o	o	o	o	o	o
Phenol „ 1-80 A.	o	o	o	o	o	o	o	o	o	o	o	o

The 30 broth tubes are arranged in a convenient rack or stand, and marked as belonging to one or other of five groups, A, B, C, D, and E, that is, each group contains six tubes. Each tube is further marked with a number signifying the contact period in minutes. Then, if a maximum 15 minutes contact be decided upon the tubes would be variously marked, A2½, A5, A7½, A10, A12½, A15, B2½, B5, B7½, and so on.

The 60 sterile watch glasses are now arranged in rows, as evident from the accompanying diagram, and divided into two groups of 30 each, namely, Group I. and Group II. Each group contains five rows of six watch glasses. Into those of Group I., sufficient disinfectant solution (of varying dilution) is placed to fill each watch glass. The rows in the group being distinguished by the letters A, B, C, D, E, from below upward. Each row of watch glasses in Group I. will contain a definite dilution of any given disinfectant. Thus, in the diagram, the lower or A row is shown to contain phenol (1 in 80), while the other rows B to E contain varying dilutions of whatever reagent is under comparison. The vertical rows of watch glasses are marked with figures corresponding to those on the broth tubes in the rack, and representing whatever period of time the thread is left in contact with the disinfectant. This is conveniently expressed in minutes.

The watch glasses in Group II. are similarly arranged, but all filled with sterile distilled water, or when working with bichloride of mercury, with sterile ammonium sulphide solution.

Single prepared threads from the Petri dishes in which they have been drying are now placed as quickly as possible in each of the watch glasses of row A in Group I. In half-a-minute from the start, or at such interval of time as has been decided upon for the experiment, other single prepared threads from the Petri dishes are placed in the glasses of B row of Group I., and so on until each watch glass in Group I. has received a thread. Assuming that the experiment has been planned for half-a-

minute interval, a period of 2½ minutes will have elapsed between the transfer of a thread into the first watch glass of row A and of one into the last glass of row E. The thread from the first glass in row A of Group I. is now rapidly transferred by means of sterile forceps to its corresponding watch glass in row A of Group II., and agitated in the water or ammonium sulphide, as the case may be, and left there. On the elapse of half-a-minute from the commencement of this transfer of thread from glass A2½ I. to A2½ II., the thread from B2½ I. is transferred to and washed in its corresponding watch glass in row B of Group II. The thread left in A2½ II. is now rapidly placed in its corresponding broth tube marked A2½. This is readily performed in the half-minute, which makes it time to transfer the thread from C2½ of I. to its corresponding watch glass in row C of II. So soon as this is done, the thread which has been left and washed in the glass B2½ of Group II. is transferred to its corresponding broth tube marked B2½. Another half-minute will now nearly have elapsed, and so soon as it has elapsed, the thread from the first glass of row D in Group I. must be transferred to its corresponding watch glass in row D of Group II. and so on. As soon as the thread in the first glass of row E1 has been washed, and that of D2½ transferred to its own broth sub-culture tube, five minutes will have elapsed from the commencement of the transfer of threads to the first glass in A1 row, and it will be time to deal with the thread in the second glass of row A1. In this manner, with half-minute intervals, the whole series of threads can be exposed to varying periods of contact in different dilutions of disinfectant, duly washed and transferred to broth for sub-culture. The interval can be made to vary according to the needs of the operator.

On completion of the whole experiment, the broth tubes, each containing a thread, are incubated at a temperature suitable for the micro-organisms employed, and the results duly recorded. In experiments with anthrax spores the incubation has been maintained for a week, with *B. typhosus* two days, and with *B. prodigiosus* five days.

This thread method presents many points of similarity in the matter of its general design to the "Drop" method as devised by Rideal and Walker. In actual practice its working is easier than the verbal description may imply.

The "Drop" method employed in these experiments is that described already by Rideal and Walker.*

In all the experiments, irrespective of method, the following conditions have been observed:—(1) The observations have been made at room

* Journal of Sanitary Institute, Vol. xxiv., 1903, p. 424.

temperature, the same being duly recorded; (2) the nutrient broth has been made invariably from chopped fresh meat with the addition of peptone and salt, the finished medium having a standard reaction of + 15 All tubes of nutrient broth contained 10 cc. of the fluid.

With the exception of experiments, Nos. 102, 103, 104, 105, 120, 130 to 140, in which 24 hour broth cultures were used, all the observations with *B. typhosus* and *B. prodigiosus* have been made by adding to the various dilutions of the disinfectant a filtered emulsion of the organism, made by scraping the growth off a 24-hour old agar slope culture and emulsifying in 5 cc. of sterile distilled water, subsequent filtration and breaking down of lumps being secured by passing through a sterile filter paper in a sterile glass funnel. In the case of anthrax cultures, these were all seven day old growths and always contained spores, as verified by microscopical examination. The subsequent preparation of the emulsion has been explained.

In the "Drop" method experiments with bichloride of mercury, the tubes of broth for sub-culture have in the majority of cases received two drops of sterile saturated ammonium sulphide solution. The addition or withholding of the sulphide to the broth in each experiment is noted in the schedule in the column devoted to method. In the same column is noted also the temperature at which the experiment was carried out.

Practically, throughout the series of "Drop" experiments, the same circular platinum loop has been used for inoculating the sub-cultures; the size of the loop being 3 mm. in diameter, and the calibre of the wire 0·4 mm.

With the exception of experiments Nos. 131, 133, and 140, in all the "Drop" method observations, the platinum needle with loop has been boldly passed into and agitated in the mixture of disinfectant and culture. In the case of the three exceptions named, the platinum loop was allowed only to drop gently below the surface of the fluid.

With the exception of experiment No. 137, one drop of infecting emulsion or broth culture has been added for each cubic centimetre of diluted disinfectant, and, as in all cases, 5 cc. of diluted disinfectant has been used, each of these tubes has received five drops of infective material. In the exception quoted, 1 cc. of the infective fluid was added to 5 cc. of the diluted reagent, and the figures in the schedule showing the dilution of the reagent employed in the experiment are those of the actual and final dilution, after the addition of the extra volume of infecting fluid.

In experiments Nos. 92, 124, 126, 127, and 128, the efficiency of the reagent in the presence of organic material was tested, by making the

required dilutions of the reagent, not with sterile distilled water only, but with four parts of sterile water and one part of sterile urine.

While fully recognising that the precise interpretation to be placed upon these scheduled experiments rests with your Committee, we venture to express our own views as to the conclusion which we ourselves think may be deduced from the work of Major Fowler.

As to the "Garnet" method, we think the results are too irregular to justify its adoption as a means of obtaining any comparative figure of disinfecting efficiency.

By the "Thread" method we are of opinion that a co-efficient or measure of comparative efficiency can be obtained, but the experiments so far made indicate that such coefficient (*qua* phenol) will be lower than that obtained by the drop method. This does not militate against its value, as this lower figure appears to be constant for all commercial disinfectants; it is a point, however, to be noted, as it is quite clear from the results before us that a given disinfectant may have say a phenol coefficient against a particular micro-organism of nine or ten by the drop method, and but one or two by the thread method. The importance of stating by what method the co-efficient has been obtained in any given case will be obvious.

We consider the technique of the "Thread" method to be so elaborate that, no matter what may be its merits, its adoption as a standard procedure seems impossible. It is eminently unfitted for working with micro-organisms at all sensitive to desiccation; see experiments Nos. 59 and 60.

In working the "Thread" method with ordinary disinfectants other than bichloride of mercury, it was found necessary to double the contact period, as will be seen by reference to experiments Nos. 26 to 60, all of which show twice the contact period as compared with experiments 16 to 25.

Provided the operator works in a room free from obvious draughts or air currents, in spite of the apparent risks of contamination by exposure of the watch glasses to the air, we find that chances of error and vitiation of sub-cultures by contamination are so remarkably small as to constitute a negligable factor.

As to the "Drop" method, we consider that, for general simplicity and facility of working, it is superior to both the garnet and thread methods, yielding in most instances results which are sharper or more definite. It must, however, be noted that even with this method difficulties are met with in securing clear and sharp results; the disturbing factors being

variations in temperature rather than variations in the strain of the same species of micro-organisms employed, and any slight differences in personal technique.

The question of temperature is very important, as the higher or lower temperature of the room in which a given experiment is carried out materially affects the efficient action of certain disinfectants, particularly phenol and the phenoloids. We anticipate some difficulty in insisting upon any precise temperature at which this class of observation should be made, but we suggest that consideration be given to the point whether a range of from 60° to 65° F. should not be laid down as desirable in standardisation experiments.

The influence of variations in strain of micro-organisms is a difficult one, and too much importance must not be attached to it, as the experiments, submitted herewith, show that the behaviour of several strains of *B. typhosus* in the presence of various disinfectants in corresponding solutions is remarkably similar. We are impressed with the fact that different strains of *B. typhosus* present certain differences as to tendency to clump, and for this reason admit some predilection for the employment of filtered cultures of this micro-organism, but the error from this source we think is small, provided that due care be taken to see that the particular strain employed is normal in all its cultural features. It is noticeable that the results with *B. prodigiosus* are largely concordant, and we suggest the advisability of your Committee considering whether it might not be advisable to employ a simple saprophytic micro-organism possessing well known stable cultural features, such as the *B. prodigiosus* as the standard against which to test the comparative efficiency of different disinfectants. The phenol coefficient of many commercial disinfectants in relation to this micro-organism appears to be nearly the same as that given when *B. typhosus* has been used in standardisation experiments.

An extended experience of the "Drop" method impresses us with the view that no coefficient should be deduced from any particular experiment unless there are at least two negative results in the phenol or comparative reagent line. Further, that before stating that any given disinfectant has a definite coefficient as against a particular micro-organism by this or any other method, the same result should be obtained in not less than three out of five observations, and with more than one sample of the reagent.

We are strongly of opinion that the practical value of any statement regarding a disinfectant would be enhanced if a duplicate series of experiments be made, using for its various dilutions not simple sterile water, but

sterile water containing some organic matter as represented by the addition of sterile urine. The importance of this point is manifest in experiments Nos. 92, 124, 126, 127, and 128, in which the phenol coefficient of certain reagents differs markedly according as to whether organic matter be present or not.

As an alternative method, we direct attention to experiments 141 to 155, in which, in place of testing the disinfectants against "naked" bacteria, that is pure cultures suspended in either sterile water or broth, the reagents have been tested against an emulsion of fresh fæces and urine. This has been prepared by rubbing up one gramme of fresh fæces in a mortar with 100 cc. of fresh urine, the whole being roughly strained by passage through an ordinary filter paper to remove clumps. This mixture contains many micro-organisms, and approximates in general character to the material against which disinfectants would be generally used in daily practice. Three cubic centimetres of this fœculent emulsion were placed in the test tubes, and an equal volume of various dilutions of the disinfectants added, the subsequent technique being that usual in carrying out the "Drop" method. The dilutions shown in the detail of experiments are those actually resulting after adding an equal volume of diluted re-agent to the fæculent fluid. Thus, where a dilution of 1 in 100 is shown, it means that three cubic centimetres of a 1 in 50 dilution of a given disinfectant were added to a similar volume of urine and fæces, the final dilution being then 1 in 100. If the results be read off and taken after 24 hours' incubation, remarkably constant and sharp results are obtainable, and quite as good as those obtained when working against "naked" bacteria. If the results be taken after a more prolonged incubation irregular readings are obtained, owing to the development of adult forms from pre-existing spores; but if the result be taken at 24 hours, the effects are recorded only so far as relates to non-sporing micro-organisms. The procedure entails little or no extra trouble, and has the advantage of standardizing the disinfectant against a natural mixture of bacteria, resembling the material against which the reagents are likely to be used in medical practice.

The evidence in favour of the superiority of phenol over bichloride of mercury, as the standard reagent for comparison, appears to us to be overwhelming.

In conclusion, we wish to place on record our appreciation of the time and labour which Major Fowler has given to this inquiry.

R. H. Firth.
Allan Macfadyen.

November 24th, 1905.